In re Appln. of Kawakami et al.
Application No. 09/898,860

SPECIFICATION AMENDMENTS
(deletions indicated by strikethrough, underlined journal titles are not additions)

Please delete the paragraph beginning at page 7, line 31.

Please delete the paragraph beginning at page 8, line 6.

Replace the paragraph beginning at page 92, line 22, with:

Peptides were a synthesized by a solid phase method using a peptide synthesizer (model AMS 422; Gilson Co. Inc., Worthington, OH) (>90% purity). The peptides to be synthesized were selected from the reported human sequence of gp100 based on HLA-A2.1 binding motifs (Falk, K., (1991) Nature 351:290; Hunt, D. F., et al, (1992) Science 225:1261; Ruppert, J., et al. (1993) Cell 74:929; Kubo, RT, et al. (1994) J Immunol. 152:3913). The following peptides were tested: Eight 8-mer peptides (with residues starting at –199, 212, 218, 237, 266, 267, 268, 269~~; see Figure 7A~~), eighty-four 9-mer peptides with residues starting at - 2, 4, 11, 18, 154, 162, 169, 171, 178, 199, 205, 209, 216, 241, 248, 250, 255, 262, 266, 267, 268, 273, 278, 280, 273, 286, 287, 298, 290, 309, 316, 332, 335, 350, 354, 358, 361, 371, 373, 384, 389, 397, 399, 400, 402, 407, 408, 420, 423, 425, 446, 449, 450, 456, 463, 465, 485, 488, 501, 512, 531, 544, 563, 570, 571, 576, 577, 578, 583, 585, 590, 592, 595, 598, 599, 601, 602, 603, 604, 606, 607, 613, 619, 648~~; see Figure 7A~~) and seventy-seven, 10-mer peptides with residues starting at – 9, 17, 57, 87, 96, 154, 161, 169, 177, 197, 199, 200, 208, 216, 224, 232, 240, 243, 250, 266, 267, 268, 272, 285, 287, 289, 297, 318, 323, 331, 342, 350, 355, 357, 365, 380, 383, 388, 391, 395, 399, 400, 406, 407, 409, 415, 432, 449, 453, 457, 462, 476, 484, 489, 492, 511, 519, 536, 543, 544, 548, 568, 570, 571, 576, 577, 584, 590, 595, 598, 599, 601, 602, 603, 605, 611, 629~~; see Figure 7A~~) were synthesized. Possible epitopes identified in the first screening were further purified by HPLC on a C-4 column (VYDAC, Hesperia, CA) (>98% purity) and the molecular weights of the peptides were verified by mass spectrometry measurement as previously described (Example 3: Kawakami, Y., et al., (1994) J. Exp. Med. 180:347; Kawakami, Y., et al., (1994) Proc Natl Acad Sci (USA) 91:6458).

Replace the paragraph beginning at page 96, line 32, with:

To complement the epitope identification using the known HLA-A2.1 binding motifs, another method was also used to identify regions possible containing epitopes. Five gp100

cDNA fragments, 4 generated by exonuclease deletion from the 3' –end of the cDNA (D3, D4, D5, C4) as well as a partial cDNA clone lacking the first 705 base pairs of the 5' –coding region (25TR), were inserted into the pcDNA3 plasmid and transfected into COS7 cells along with the HLA-A2.1 cDNA. ~~The locations of the fragments are shown in Figure 7a.~~ The recognition of these transfectants by the 4 gp100 reactive TIL was evaluated using an IFN-γ release assay ~~(Figure 7B)~~. TIL1200 recognized COS cells transfected with the fragments, 25TR, D5, D4, or C4, but not with D3, suggesting that at least 2 epitopes existed in the regions of amino acid residues 146-163 and 236-661. $G9_{154}$ and $G10_{154}$ were the only peptides which contained HLA-A2.1 binding motifs in the region 146-163 and both were recognized by TIL1200. G10-4 was located in the region 236-661 and was recognized by TIL1200. TIL620-1 recognized COS cells transfected with C4 but not with D3, D5, D4 or 25TR, suggesting that the epitope existed within residues 187-270. $G9_{209}$ and $G10_{208}$ which were recognized by TIL620-1, were located in this region. TIL620-2 another subculture of TIL620, also recognized COS cells transfected with D5 and D4, but not D3, and recognized $G9_{154}$ and $G10_{154}$ in the region 147-163, also recognized by TIL1200. TIL660-1 and TIL1143 recognized COS cells transfected with C4 or 25TR, but not with D3, D5, or D4, suggesting that epitopes existed in the 2 regions 187-270 and 236-661. $G9_{280}$ located in the fragment 25TR, but not in the fragment C4, was recognized by TIL660 and TIL1143.

Replace the paragraph beginning at page 97, line 31, with:

With the exception of G10-4, which required a concentration of 1ug/ml to sensitize T2 cells for CTL lysis (Example 3; Kawakami, Y., et al., (1994) *Proc Natl Acad Sci* (USA) 91:6458), all gp100 epitopes identified in this study could sensitize T2 cells for CTL lysis at a concentration of 1ng/ml ~~(Figures 8A-8D)~~. G10-5 appeared to be inhibitory to the cytotoxic activity of CTL at concentration greater than 10ng/ml since lysis of T2 cells incubated with G10-5 at more than 10ng/ml was repeatedly lower than at 1-10ng/ml in this assay condition in which the peptide was present in the medium during entire 4h cytotoxicity assay ~~(Figure 8D)~~. The relative binding affinity of these epitopes to HLA-A2.1 was also measured using an in vitro competitive binding assay (Table 13). $G9_{154}$, had an higher binding affinity (50% inhibition of the standard peptide at 11nM) to the HLA-A2.1 molecule than $G10_{154}$ (1010nM) which contains an extra leucine at the C-terminus of $G9_{154}$, and could sensitize T2 cells at lower concentration than $G10_{154}$ ~~(Figure 8A)~~. $G9_{209}$ also bound to HLA-A2.1 with higher affinity (84nm) than $G10_{208}$ (2080nM), which contains an extra threonine at the N-terminus, and could sensitize T2 cells at lower concentrations of peptide than $G10_{208}$ ~~(Figure 8B)~~.

Thus, the 9-mer peptides were superior to the corresponding 10 mer peptides in the sensitization of T2 cells to CTL lysis, and they also had higher binding affinities to HLA-A2.1. This was also the case for the identified MART-1 9 and 10 amino acid peptides (M9-2, M10-3, M10-4) (Example 2; Kawakami, Y., et al., (1994). *J. Exp. Med.* 180:347). The results of the peptide titration in the T2 cell lysis assay correlated with the results of the HLA-A2.1 binding affinity as measured by the in vitro binding assay. The other gp100 epitopes, $G9_{280}$, and G10-4 or G10-5 had binding affinities for HLA-A2.1 with 50% inhibition at 95nM, 483nM, or 13nM, respectively. The HLA-A2.1 binding affinities of the previously identified HLA-A2 restricted melanoma epitopes in MART-1 (Example 2; Kawakami, Y., et al., (1994) *J. Exp. Med.* 180:347) and tyrosinase (Wolfel, T., (1994) *Eur J. Immunol.* 24:759) were also measured (M9-2 (397nM), M10-3 (2272nM), M10-4 (5555nM), T9, (333nM), $T9_{369}$ (40nM)). Except for the 10mer peptides ($G10_{154}$, $G10_{208}$, GM10-3, GM10-4), for which overlapping 9-mer epitopes ($G9_{154}$, $G9_{209}$, M9-2) existed, all melanoma epitopes had either high ($G9_{154}$, G10-5, $T9_{369}$) or intermediate ($G9_{209}$, $G9_{280}$, G10-4, M9-2, $T9_1$) binding affinities to HLA-A2.1.